A SURVEY OF METHODS USED IN THE STUDY OF MICROFUNGAL SUCCESSION ON LEAF SURFACES

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The fungi on leaf surfaces have been investigated by a variety of techniques which fall into two groups; studies in which fungi are observed directly on the plant surface and others where fungi are encouraged to grow and sporulate on suitable media under appropriate environmental conditions. The use that has been made of these techniques is shown in Table I where the techniques are grouped into direct and cultural studies and are listed within these groups in chronological order of use from left to right. The table only includes studies directly concerned with successional studies and does not necessarily indicate the origins of the techniques used.

Direct observation has only been recorded when fungi have been searched for without prior treatment of the material. Leaf washing has been recorded when it has been used to identify loosely-attached propagules and fungi and not merely as a preparatory treatment. Washing with added surfactants has not been distinguished from ordinary washing. Surface sterilisation has only been recorded when it has been used to differentiate between surface and internal fungi. Damp chambers have been used in two ways. A short period of incubation (2 days) has been advocated to encourage the sporulation of fungi already growing on the leaf surface (Webster, 1956). A longer period (7-28 days) enables fungi originally present as propagules to sporulate. None of the authors listed in Table I recorded observations at both two and more than seven days, although these may well have been made. Techniques using these two periods have, therefore, been differentiated in the table.

Table 1. Techniques used by various authors in analyses of fungal successions on leaf surfaces.

		direct			cultural									
		direct observation	impression films	clearing	scanning microscopy	spore fall	plating	damp chamber (2 days)	damp chamber (7 days)	surface sterilisation	leaf washing	leaf maceration	leaf impressions	no. of techniques used	mean no. of techniques/study
1955-1959	Last, 1955					x								1	1.8
	Webster, 1956							x						1	
	Hudson and Webster, 1958						x	x						2	
	Pugh, 1958						x			x	x			3	
1960-1964	Ruinen, 1961	x	x				x		x					4	2.6
	Hudson, 1962							x						1	
	Kendrick and Burges, 1962	x					x			x	x			5	
	Meredith, 1962							x						1	
	Kerling, 1964										x			2	
1965-1969	Dickinson, 1965					x	x				x			3	3.0
	Hogg and Hudson, 1966					x	x	x						3	
	Dickinson, 1967		x				x		x		x			4	
	Pugh and Williams, 1968						x				x			2	
	Hislop and Cox, 1969					x					x	x		3	
1970-1974	Lamb and Brown, 1970			x		x							x	3	4.0
	Pugh and Buckley, 1971			x	x	x	x			x	x			6	
	Pugh and Mulder, 1971						x		x	x	x			4	
	Ruscoe, 1971	x				x	x		x	x				5	
	Apinis *et al.*, 1972					x			x		x		x	4	
	Bainbridge and Dickinson, 1972		x			x	x		x		x		x	6	
	Norse, 1972		x			x								2	
	Dickinson, 1973			x		x					x			3	
	Lindsey, 1973	x	x	x	x	x	x		x	x	x		x	10	
	Godfrey, 1974			x			x							2	
	Sherwood and Carroll, 1974	x												1	
	Diem, 1974		x								x			2	

In general there has been an increase in the number of techniques used in each study. It has long been recognised that individual cultural methods tend to favour certain groups of fungi (Dickinson, 1971). For instance, the spore fall method is suitable for the detection of the so-called "shadow yeasts" but not for the filamentous fungi which grow readily from leaf discs plated on nutrient media. A growing appreciation of the selective aspects of techniques is probably responsible for the use of an increasing variety of techniques over the years. However, some studies have been made of succession in a narrow range of organisms (e.g. Last, 1955) and others have been of refractory materials (e.g. Hudson, 1962) and in these cases there has either been no need of, or opportunity for, the use of several techniques. The merits and demerits of cultural techniques commonly used in phylloplane studies have been reviewed by Dickinson (1971).

Techniques used in early studies were mainly developed from methods used in the study of soil and litter fungi and hence, the emphasis is on a cultural rather than direct approach. Since 1960 there has been an increase in the use of direct techniques. These have usually been allied with cultural techniques. This combination has been advocated by Dickinson (1967, 1971) and Last and Warren (1972) among others, and was used by Pugh and Buckley (1971) to show that *Aureobasidium* grew on *Acer* leaves only until the arrival of *Cladosporium* and *Epicoccum* when it formed chlamydospores. The recent use of relatively sophisticated direct techniques in studies mainly concerned with the distribution of fungi on leaf surfaces has indicated that the common primary saprophytes isolated by cultural methods may be less important on the leaf surface than yeasts and yeast-like fungi (Dickinson, 1967; Diem, 1974). However, so far, there have been few published studies of the succession of these micro-organisms which is probably due to problems of identification. Warren (this volume) has demonstrated fluctuations in the populations of white yeasts on leaf surfaces using a pour plate technique.

Future successional studies of phylloplane fungi will probably use balanced combinations of direct and cultural techniques designed to investigate different but, probably, overlapping components of leaf surface populations.

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